REMARKS

Please enter the above before consideration of this application. With respect to the above newly entered claims, the subject matter of the claims is editorially revised and rewritten to bring that subject matter into conformity with the United States claim format.

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Respectfully submitted,

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[012] Thus, although quantitative and extremely sensitive methods for DNA/RNA sequencing exist, these methods are time consuming, require painstaking sample preparation and expensive equipment, and are generally not available as portable systems.

[013] **DETAILED** DESCRIPTION OF THE INVENTION

[014] Therefore, it is the object of the present invention to create for detecting nucleic acid oligomer hybrids an apparatus and a method that do not exhibit the disadvantages of the background art.

[015] According to the present invention, this object is solved by the modified nucleic acid oligomer ~~according to independent claim 1~~, the method of producing a modified nucleic acid oligomer ~~according to independent claim 21~~, the modified conductive surface ~~according to independent claim 29~~, the method of producing a modified conductive surface ~~according to independent claim 44~~, and a method of electrochemically detecting nucleic acid oligomer hybridization events ~~according to independent claim 48~~.

[016] The following abbreviations and terms will be used in the context of the present invention:

[017]

Genetics

DNA	deoxyribonucleic acid
RNA	ribonucleic acid
PNA	peptide nucleic acid (synthetic DNA or RNA in which the sugar-phosphate moiety is replaced by an amino acid. If the sugar-phosphate moiety is replaced by the -NH-$(CH_2)_2$-N($COCH_2$-base)-CH_2CO- moiety, PNA will hybridize with DNA.)
A	adenine
G	guanine
C	cytosine

that, due to its being shaped as a cut-off cone that is hollow inside, coats a cyclophane or similar electron-donor/electron-acceptor complex.

[055] According to the present invention, a redox-active moiety is covalently bound to a nucleic acid oligomer by the reaction of the nucleic acid oligomer with the redox-active moiety or portions thereof (see also the section "~~Manner~~Detailed Description of ~~Executing~~ the Invention"). This bond can be achieved in four different ways:

[056] a) A free phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine group of the oligonucleotide backbone, especially a group at one of the two ends of the oligonucleotide backbone, is used as the reactive group for forming a bond at the nucleic acid oligomer. The free, terminal phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine groups exhibit increased reactivity and thus easily undergo typical reactions such as amidation with (primary or secondary) amino groups or with acid groups; esterification with (primary, secondary, or tertiary) alcohols or with acid groups; thioester formation with (primary, secondary, or tertiary) thioalcohols or with acid groups, or condensation of amine and aldehyde with subsequent reduction of the resultant CH=N bond to a CH_2-NH bond. The coupling group (acid, amine, alcohol, thioalcohol, or aldehyde function) required to covalently attach the redox-active moiety is either naturally present on the redox-active moiety or is obtained by chemically modifying the redox-active moiety. The attachment of the redox-active moiety may take place completely or in portions of the moiety with subsequent completion of the redox-active moiety (see below).

[057] b) The nucleic acid oligomer is modified with a reactive group at the oligonucleotide backbone or at a base via a covalently-attached molecular moiety (spacer) of any composition and chain length (longest continuous chain of atoms bound to one another), especially a chain length of 1 to 14. The modification preferably takes places at one of the ends of the oligonucleotide backbone or at a terminal base. An alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl substituent, for example, may be used as the spacer. Possible simple reactions for forming the covalent bond between the redox-active moiety and the nucleic acid oligomer thus modified are, as described under a), amidation from an acid and amino group, esterification

amine group can react directly with the unmodified surface, as described under b) in this section. In addition, a further reactive group may be bound to the oligonucleotides near their second end, this reactive group, in turn, being attached, as described above, directly or via a spacer of any composition and chain length, especially a chain length of 1 - 14. Furthermore, as an alternative to this further reactive group, the redox-active moiety (completely or portions thereof) may be attached to this second end of the nucleic acid oligomer.

[072] Binding the nucleic acid oligomer to the conductive surface may take place before or after the redox-active moiety is attached to the nucleic acid oligomer. In the case of a redox-active protein/enzyme comprising apoprotein and cofactor(s), instead of the complete redox-active moiety, it is also possible for only the apoprotein, the apoprotein and a portion of the cofactors, or one or more of the cofactors to be attached and the redox-active moiety will be completed by subsequent reconstitution with the remaining missing portions. If a linked (at least bimolecular) electron-donor/electron-acceptor complex is used as the redox-active moiety, the electron acceptor (or donor) may, as described under b) or c) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," be attached to a terminal base, or in place of a terminal base, to the nucleic acid oligomer, and the electron donor (or acceptor) may be attached by subsequent covalent attachment to a reactive group of the electron acceptor (or donor) or, as described under a) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," by subsequent attachment to a terminal reactive group of the nucleic acid oligomer backbone at the same end (see also the section "~~Manner~~**Detailed Description** ~~of Executing~~ the Invention"). Alternatively, binding the nucleic acid oligomer to the conductive surface may take place before or after the spacer having a reactive group for binding the redox-active moiety is attached. Binding the already modified nucleic acid oligomer to the conductive surface, i.e. binding to the surface after the redox-active moiety is attached to the nucleic acid oligomer or after portions of the redox-active moiety are attached, or after the spacer having a reactive group for binding the redox-active moiety is attached, likewise takes place as described under a) to c) in this section.

strand oligonucleotide is used, the oligonucleotide double-strand is thermally dehybridized after the double-strand oligonucleotide is attached to the surface.

[077] Regarding the individual steps in "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," as well as in "Binding an Oligonucleotide to the Conductive Surface," it should be noted that, in the section "~~Manner~~**Detailed Description** of ~~Executing~~ the Invention," the various combination possibilities of the individual steps that lead to the same end result are demonstrated in an example (Figure 2).

[078] **Method of Electrochemically Detecting Nucleic Acid Oligomer Hybrids**

[079] Advantageously, according to the method of electrochemically detecting nucleic acid oligomer hybrids, multiple probe nucleic acid oligomers varying in sequence, ideally all necessary combinations of the nucleic acid oligomer, are applied to an oligomer (DNA) chip to detect the sequence of any target nucleic acid oligomer or (fragmented) target DNA, or in order to seek and sequence-specifically detect mutations in the target. For this purpose, the surface atoms or molecules of a defined area (a test site) on a conductive surface are linked with DNA/RNA/PNA nucleic acid oligomers having a known but arbitrary sequence, as described above. In a most general embodiment, however, the DNA chip may also be derivatized with a single probe oligonucleotide. Preferred probe nucleic acid oligomers are nucleic acid oligomers (e.g. DNA, RNA, or PNA fragments) of base length 3 to 50, preferably of length 5 to 30, particularly preferably of length 8 to 25. According to the present invention, a redox-active moiety is or becomes bound to the probe nucleic acid oligomers, as described below.

[080] The modification of the probe nucleic acid oligomers with a redox-active moiety may take place completely or in components of the redox-active moiety, either before or after the oligonucleotide probe is bound to the conductive surface. The various combination possibilities of the individual steps (reaction sequences) are demonstrated in the section "~~Manner~~**Detailed Description** of ~~Executing~~ the Invention" with the aid of Figure 2 using the example of a redox-active moiety bound to an electrode via a probe oligonucleotide.

potential at which the direct reaction of the free redox-active substance with the electrode can be significantly suppressed, and primarily electron transfers between the redox-active moiety and the electrode can be detected.

[090] If the redox-active moiety is a photoinducibly redox-active moiety, the redox activity of the moiety is triggered only by light of a specific or any given wavelength. According to the present invention, this property is used to advantage in that electrochemical detection is triggered only by radiating light onto the surface hybrid having the general structure elec-spacer-ds-oligo-spacer-moiety (surface hybrid with hybridized target) and is maintained, at most, as long as light irradiation continues. Thus, particularly in the case of amperometric detection, if a photoinducibly redox-active moiety is used, under certain external conditions, (rather long-lasting) current will flow only if light is radiated onto the surface hybrid. Such external conditions are for example the presence of a reducing agent (or oxidizing agent) suitable for reducing (or oxidizing) a photoinductively-formed oxidized donor D^+ (or reduced acceptor A^-) of the photoinducibly redox-active moiety, and applying to the electrode a potential at which a photoinductively-formed reduced acceptor A^- (or oxidized donor D^+) of the photoinducibly redox-active moiety can be oxidized (or reduced), but the non-reduced acceptor A (or the non-oxidized donor D) cannot be oxidized (or reduced). In the section "~~Manner~~**Detailed Description** of ~~Executing~~ the Invention," this is explained in greater detail using various examples of an elec-spacer-ss-oligo-spacer-moiety having a photoinducibly redox-active moiety. In this way, detection using a photoinducibly redox-active moiety can be spatially limited to a certain test site or group of test sites of the oligomer chip by restricting the light to this test site or group of test sites. According to the present invention, various test sites (nucleic acid oligomer combinations) of an oligomer chip can thus be applied to a shared, continuous, electrically-conductive surface. A particular test site or group of test sites can be addressed and amperometrically detected simply by applying a suitable external potential to the (entire) surface if precisely this test site or group of test sites is irradiated with light. The various test sites thus need not be applied to individual (micro-) electrodes that are electrically isolated from one another and individually controllable for applying a potential and reading out the current. Moreover, if surface hybrids having the general structure elec-spacer-ss-oligo-

[098] Fig. 6 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-Q-ZnBChl of Figure 5 having gold as the solid support material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH- as the spacer between the electron acceptor PQQ and the oligonucleotide, as well as a diagram of the sequence of the photoinduced electron transfer steps. The 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' in the hybridized state is shown in detail.

[099] ~~MANNER~~DETAILED DESCRIPTION OF ~~EXECUTING~~ THE INVENTION

[100] A formation unit of an exemplary test site with hybridized target, Au-S$(CH_2)_2$-ds-oligo-spacer-UQ(RC) having the general structure elec-spacer-ds-oligo-spacer-moiety, is illustrated in Figure 4. In the context of the present invention, "formation unit" is understood to mean the smallest repeating unit of a test site. In the example in Figure 4, the surface is a gold electrode. The link between the gold electrode and the probe oligonucleotide was formed with the linker (HO-$(CH_2)_2$-S)$_2$, which was esterified with the terminal phosphate group at the 3'-end to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH and, following homolytic cleavage of the S-S bond at the gold surface, produced one Au-S bond each, with which 2-hydroxy-mercaptoethanol and mercaptoethanol-bridged oligonucleotide was coadsorbed on the surface. The photoinducibly redox-active moiety in the example in Figure 4 is the reaction center (RC) of the photosynthesizing bacteria *Rhodobacter sphaeroides*, a photoinducibly redox-active protein consisting of apoprotein and cofactors. In the application example, the RC, via its cofactor ubiquinone-50 (UQ) in what is known as the Q_A binding pocket of the RC, is covalently joined with the oligonucleotide, where free UQ was first provided with a reactive carboxylic-acid group (see Example 1), then covalently attached to the probe oligonucleotide via this carboxylic-acid group (amidation and dehydration of the terminal amino function of the -CH=CH-CO-NH-CH_2-CH_2-NH_2 linker attached at the C-5 position of the 5' thymine),